

Discrepancies in b/i ratios.

1000

"99/CIC .. seems to point to a bias among i:b transductions correlated with easiness of swarming.

Hyp.

1. i's start earlier for unknown reasons, but move at same rate as b
2. transductions start at the same time, but there are differences in effective motility
- 3B. Following selection which results in comparable rates!

Repels unpurified susp of 99/CIC:

1001 A 1-3 = CIC early swarms, i

B 4-7 = CIC delayed i

C 8-14 = CIC delayed b.

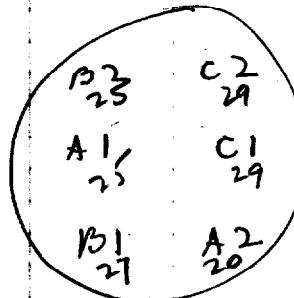
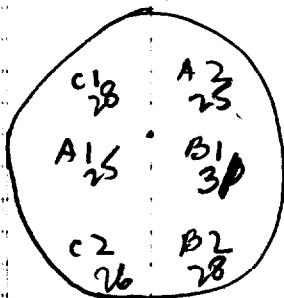
Lysogenicity/LT2.

all +

all +

#1? 2,3,4,7+ (5,6 -)

From suspension motility, compare movement or not. agar 2P11-8, 11 duplicate plates, 2 from each.



- dear m. miss

A 25-25 25-20

B 31-27 28-25

C 28-29 26-29

If anything, B, C > A rather than the converse! These were isolated from a different plate, and any difference may be irrelevant to the present issue. Hyp. 2 maybe excluded, but 1 vs. 2B cannot now be determined.

But see also 1030.

ca 12/5/52 | Prepared by M.L. More
Label as no.

- 922 Lw 1 ♂ had some Fla. + activity in SW666.
 927 Lw 1 ♂
 928 Lw 1 no Fla. + as expected.

~~some~~
some change.

Titrate 928 on SW666, 922 and 927 on LT-2.

922 $\leq 10^7$

927 $\leq 10^7$

928 412×10^7 tit. 10^{10} by long del.

unfortunately, few suitable

12/12	927 Lw 2. 94×10^6 /LT2	Galt/666 /ml	swarmes
	999-16 = SW944	Lw. HLM. ca 10^{10} ; 40×10^8	625 +++
	999-24		
	999-66	$\leq 10^6$ but many minute plaques!	
	SW686	ca 10^{10} 30×10^8	397 +++

Retitrate it

A. SW927 No UV

in SW950.

B. 2×10^5

200×10^5

C. 10^6

100×10^7

D. No UV as A

10^5

E. No UV Sup't and end of
Try Taylor doses $24-48$ hr. culture

10^4

$3 \times 10^5 \pm$

(927 Lw 3)

Respectable lysis!

) plates covered;
counts $\pm 100x$

Serum inactivation

1003

12/12/52

Shear's anti FA serum.

Add .1 ml to 10 ml ca. 10^8 PLT2 in broth. Incub. at 37° 10 minutes.
 Assays on LT-2.

	control	serum 82	∴ ca 2 decades in 10m.
3	++		
5	182	3	at 1:100
6	21		∴ k = ca 50/minute.

poor
counts

Do a more careful test in this range:

Quantity of serum needed to prevent plaquing (and presumably, secondary lytic conversion).

12/19. Mix ca. 1 ml 2×10^6 PLT2 + .1 ml 10^{10} LT-2 + 10 ml ~~broth~~

After 15 minutes add to equal volume of various serum dilutions, plate on E14B lac.

1	1:1	plaques erratic, small, may be reduced in number.	
2	1:5	ca 10^{3+}	
4	1:10	"	Note: clear plaques noted: 3 or "0" 1 or "2" no others!
8	1:20	"	
16	1:40	"	Very high serum conc. will be needed to prevent
32	1:80	"	cross-infection!
64	1:160	"	
128	1:320	"	
256	1:640	"	
0	0	ca 10^{3+}	

Copper / Yeast

100%

Dec. 15, 1952

12/14. More ca 5×10^7 cells in 10ml D(0) + Cu.

CuSO ₄	A			B		
	a	b	c	a	b	c
1 0	+++	+++	+++	+++	++	++
2 5×10^{-6}	+	++	+++ 10% min	+	++	++
3 10^{-5}	+	++	++	+	++	+++ 10% min?
4 2×10^{-5}	±	+	++ or min?	±	+	++
5 10^{-4}	±	+	++	±	+	++

6PM. 1st reading 9:30 AM 12/15.

- a) streak out on N.A. 9:30 AM. fermentate.
- b) 1:10 PM
- c) 6PM 12/16.

Results 10D4 A2-4, B63 — normal size colonies in both.

c: few smaller colonies in B5, A2, B2, A3, A4. Results \rightarrow (normal size)

12/18. Repeat expt. in T(0) colonies as above

53

CuSO ₄	1485 48h			B		
	colonies			8	9	10
1 0	+++	++	++	+++	++	++
2 5×10^{-6}	+	++	++	+	++	++
3 10^{-5}	-	+	few ++	-	++	++
4 2×10^{-5}	-	-	-	-	-	-
5 10^{-4}	-	-	++	-	-	-
6 D(0) 10^{-5}	+	±	-	-	-	-
7 D(0)	+++	++	++	+	+	++

No dwarf colonies from any plating!

Streak out at cg 286 no growth!
Results 48h.

(D(0)) "extate? - detoxified?"

Reheats W1939:

	T(0) colonies	T(typ)	D(0)
1939	+	++	+
1939A	+	++	+
2042	+++	++	" ++
2090	++	++	++

D(typ) ^{Perm} AB
++ ++ 17

14 ++ ++ ++ ++

15 ++ ++ ++

16 + ++ ++ ++

Note almost = growth 18-20 on trypt. Gause Nutr. Both from Pennsylvania;
streak out. Note lack of stain. key tryptophane for 2090. 2090 seems to
do relatively much better in both than on agar! (over)

Try β -12 on broth, agar. No effect.

No S_2S_{203}
2090 - colonies sl. less turbid than the others in Penassay.

cf. aerated and un-aerated Penassay 2090, 1939A: 2090 Aer: increased
intensity at same slow rate as 1939A
but reached higher final level.

despite poor growth use inoculum from C2 for MNQ expt.

in T(0) 4P19 E. coli B + W1939A, inoc. per Colwell

D) 25, 12, 6, 3 ppm MNQ to T(0) liquid.

	"1939A"	"B"
MNQ	0 tubedown +++	36h. tubedown.
PPM	3 +++	++
	6 ++	++
	12 +++	- normal colonies only.
	25 - n.g.	- ++
		- +

12/21. Restraints. A25, B

A25: sterile

B25, 12 normal ++ only

B25 some minute colonies
+ numerous ++.

Restraint these.
all normal size.

These expts. fail to confirm Weed, Colwell and co-workers

of 1951

To received from Colwell (1st and 2nd time) W1939A is a mixture of
Mal+ and Mal-. Both are highly stable, but Mal+ after 2 yrs. did give
a few papillae \rightarrow Mal+ and +. (Mal+) + W2090 did not give normal Mal-.

Strains for phase control.

1005

S. Sendai sw 771 is being considered for ~~the~~ studies on determination of phase. However, it appears to be mainly rough. Attempt to isolate a smoother strain.

All isolates, incl. from lymphoid, are at least partly rough in habit on nutrient agar, rendering H-scoring difficult. Bradolites; H- is rapidly attenuated on solid medium. Ditto for sw 925.
Look at other *Salmonella* diphasics

see 1024, 1035

Multiplicity reactivation

1006

FA12: ca 10^9 E3: "160/ml". Does this depend on multiplicity?

SW666 ~~12/17~~. Estimated bacteria = 2×10^{10} FA12 $\approx 10^9$

① Mix .1ml E3 + .1ml undiluted bacteria. Plate .1ml on EMB Lac + ~~Ford undilute~~

② " " bacteria 1:10

+ ~~.1ml undilute~~

③ " " " 1:100

+ ~~.1ml undilute~~

ND M W
by wt.

After 10 mins RT. add .1ml saline to ①, .1ml undilute to ②, ③ and plate .1ml samples on EMB Lac., afterthought also add EMB Lac Cal: plaques. Cal +

1	6,7	(.075ml)	2
2	2,8	(.05ml)	4
3	1,5	(.06ml)	1

Multiplicity reactivation probably does not account for the residual plaques.

"lytic variant"

1003

12/22

See 1003: streak out plaques 1-4 to isolate pure lines. An initial streaking

#1 was mix. of clear and turbid plaques, both small; 2+3 also mixed, mostly larger clear plaques, 4 small and large clear plaques. For fast phys. pick well isolated clear plaques, grow on LT-2

In pul tests, 927 and 928 was not lysed by any plaque. LT-2 was grossly lysed. 666 showed only few weak plaques on #4.

Grow plaques on LT-2/2 in broth. All but #3 gave v. clear lysates.

- a) test vs LT-2, 927 etc. (1/2 dil.)
 b. Add ca 10^5 cells LT-2 to eq. vol ~~22V~~ LT-22. Plate in .1 ml 22V4
 at various intervals, incl. 0. Then there is a final plating
 $\approx \frac{1}{4} \times .1 \text{ ml}$ of diluted cells. All platings give volumes

1	No FA22, No 22V	ca. log phase. 10^3	$\frac{1}{2} \text{ ml}$
2	No FA22, 22V	(1 or 2)	1 minute exp. to 22 protots
3	60M FA22	10^3	against 22V! Decrease of
4	" + 22V	10^3	very high multiplicity of 22, this
5	1M FA22 + 22V	10^3	may be adsorption - interference!
6	25M FA22 + 22V	10^3	
7	25 " , no 22V	10^3	
8	45M FA22	10^3	
9	45M " + 22V	10^3	
10	100M		

Titer of V4: 12×10^9 , $++ \times 10^7$ confl. 10^5 . All plaques clear!
 (For more accurate titration, use 10^8 .)

Serial dilutions	V:				\therefore can choose serial to subl rough.
	1	2	3	4	
LT2	S	S	S	S	
927	R	R	R	R	
666					
928	R	R	R	R	
912	S	S	S	S	
688	R	R	R	R	
(over)					

some "smooth" strains of ~~22V~~ 22V + LT2.

Follow 4 through serial streaking:

gave self-mixed~~ed~~ streaks until isolated
as PLT-22, 22V - sensitive, non-lysogenic.

On SW666 22V gave scattered plaques at 10^{-3}

(e.g. ca 10^{-5}). Plaques not very sharp, not lytic.

Restreak on SW666 to get 22BV - retains lysis action on LT2,
gives turbid plaques on SW666.

However, attempt to reduce lysogenicity in SW666 gave 4/4 still sensitive

10^{15} ml. 1.2 ml autg. $10^3, 10^6, 10^8$ PLT22 + 10^9 LT2/ml (2ml)
RT.

	PLT22		
A	10^8 count: 111×10^7	+ V	3×10^7 288×10^5
B	10^6 count: 120×10^7	+ V:	0×10^7 22×10^5 (589×10^3) sic!
C	10^3 plaques: 23×10^6 (smear) with V: $+++ \times 10^6$		
D	0 count: 129×10^7 , with 22V: 6×10^5	1073×10^3	$++9 \times 10^6$

Plate C 22V at various dilutions. also filtration C.

Stock PLT22 assumed 3×10^{10} if plaque titre and "protective titre".

Results. Phage added was 2.3×10^8 initial rather than 10^7 .

Bacterial count was 1.2×10^9 containing 1.07×10^6 background resistant.

Phage added	V22 ^K recovered
2.3×10^7	$+ 2.78 \times 10^5$
2.3×10^5	$10+12 \times 10^5$
2.3×10^4	—
0	6×10^5 10×10^5

$\therefore \frac{2.78}{2.3} = 1.2$ resistant bacteria per plaque-former!

7 contaminated colonies appear at A ($\times 10^{-7}$) autg. for all added units!

B Test transduction of FA Y3 (22VY). 1ml

sw935 D(8) 0/8 salt + 10

sw928; no success

all salt stable. Test by sphaericity after

centrifugation: all lysogenic possibly exc. #1a (grows poorly!)

This is neither lysog. nor sensitive

Type of lysogenicity:

Kill reosp. in CHCl_3 , streak on LT-2

1 22	7 ~
2 "	8 22
3 "	9 "
4 "	10 "
5 22	
6 ~	

> 2 types is
turbid plaque

6, 7 need streak more in hemagglutination on LT-2

✓ 6, 7 → turbid plaques.

FA43 → LA22.

1006E

10 salt + transductions. Restained and replicated to LT 2.
All strains are lysogenic. #1 and 2 had possibly non-lysogenic
components : E2 lysogenic

E1 appears to be non-lysogenic; resistant to 22, 22V.
It still reacts with anti IX-XII. May be an
"immune - 1" type. Test for transducibility.

By growing E1 & LT2, turbid plaques were seen.

1006E1 may thus be another "weak lysogenic": y.

1009B4, 1009C1.

1006E1 plated alone gave numerous small colonies

D(a) + FA43 gave 1 large + " "

+ FA22, 1 ml ca 300+ larger colonies

435 + FA43 : 1, 3, 5

435 alone gave 6 !

Do SW435 now
spont. - unreliable?
May be "intermediate" allele

Resuscitate, small colonies may be living on added broth carryover.

Check these colonies (5 each)

		Gal -
1	SW435 sp.	++ ++ ++ ++ ++
2	1006E1 sp.	++ + + ++ ++
3	1006E1 FA43	+++ +++ ++ ++ ++
4	" FA22	+++ +++ ++ +++ ++
5	SW435 FA43	++ ++ * ++ ++

++ strong growth in 24h.

++ " " 48h., mil broths. (supernatant?) (over)

+ moderate "

Apparent reversion may be weaker (correspond to intermediate?)

SW435 now may be unreliable. Check agglutinins: reisolate if nec. Compare
240, 279

G. supernates of
1006E1, SW 435
= 1006E1A 1006E1C

X-ray of 22B

1007

12/23/52.

X-ray counting A Morris / 4 of the day

SW 686 hr A note B 100,000²(pw. fac. 3×10^9)C 200,000² O: original stock

$\varrho \times 10^7$	Repeat	Cal+ / 666 .1ml	Cassay on large plates $\times 10^7$
N 9. A 92	53,61 .57	36	61,70
S 10. B 26	24,31 28	48	**
C 117	3,7 4	12	(7.3; 6,2) 6.8 (7.3×10^{-6})

original stock. 64

Repeat
any how shows
v. little
effect of
v. v. v.

~~approximately 1 decade per 10%.~~

$\therefore Q$ is attenuated ca 1 decade / $200,000$
FA is diminished (?) from 26-12

In view of tremendous doses required, this avenue does not appear to be promising

C strayed out: all stable salt+.

Incidence of heterogeneity: 10/12

ca 12/10/53

SW 948-949.

+ FA 9, 10, 12 gave no Tox S except
1 extensive tract 12 → 948
a few swarms? 10 → 948

FA 10 → 948 2b, 2a FA 22 → 948 2a.

SW 948 grows smoothly in broth but gives very rough colonies on
(?) NSA. Nevertheless, these react strongly with anti-I-II; ~~IX-XIV~~
check absorption of PLT-22. cf 925

see 1045

Where are results of 22 → para A-O?

Dec. 24, 1952

11:35 PM.

LT-2 PL/22 dil to "10⁹".

Total ($\times 10^7$)
117,134

Contaminated.

A B: count

^{Worn along.}

B B/1ml + 4/1ml 78,78 (sic)

C B, 1ml + 4/1ml 85,88

D plaque assay (Gratiophiles: sal susp LT-2 $\times 10^7$: 67,73. i push cell dil in terms of original B cell.
^{"10⁹ phase}

conditions of cells may affect the quantitative recovery of 4 (as there may be dead cells or other inhibitors)

No effect of multiplicity on survival. The actual ratio 4/B was
~~125/78~~ in B and 130/

130/125 in B and 130/82 in C but amount actually adsorbed was not established.

Detail in B, C:

$i \div 10^9$ C. 85 plating. Obviously contaminated. Many additional are vaguely mottled. Estimate these. Replicate to 22V. Central plaque general.

C. 88 Replicate to ~~LT-2~~ LT-2 4 contam.

B ($r \approx 1$) Many colonies have a central clearing, difficult to tell whether definite plaque or not. obvious plaque in 17/78; almost all others have a central plaque.

recover

Small central plaque are multiply infected?
Large plaque & lysed are singly??

1009181
Total 78 Test lysogenicity by replication

B: lysogenic

all non lys. were more or less
lysed by 22V.

21 non-lysogenic

57 lys. of these, 17 showed obvious plagues and are therefore
contaminated.

On replication, 8 additional were ~~soft~~ sector-plaged
strains out remaining lysogenic for purity (15 can be picked)

25 contain. or colony 27 not

21 non-lysogenic

15 12 not adequately tested or lysogenic
picked or not platable.

$$\frac{21}{78} \text{ non lysogenic} = .269 = e^{-1.3}$$

$$\text{actual ratio } B:\varphi = \frac{120}{125} = 1.04$$

$\varphi:B$
C: ~~A~~ ca 10.

$$C: 7/88 \text{ non-lysogenic} = .0795 = e^{-2.5}$$

Are these non-lysogenized? Test for sensitivity ($22V^s$)

also test "non-contaminated" lysogens.

of remaining 81, only 4 were obviously contaminated

A: non-lysogenic on PLT22

B strains out "non-contaminated" lysogens

In preliminary test of C1, 8 colonies were picked (7 resist 22V)

All showed a few lysed colonies on strain. Conclude that
all are actually contaminated.

12/25/26

dilute stock FA22 at 1:30

B UV A 20 minutes (10ml) at 50 cm.

Add 1ml to 1ml LT2 (10 hour broth)

Plate out & 22V. at 10^{-5} , ~~10⁻⁴~~O: LT2 is FA22 + 22V, 10^{-5} : 5 survivors.(A) G. LT2 & FA22 ~ 10^3 plaques on LT2; ~ 10^3 survivors 22V at 10^{-5} (B) H. plaques: 0×10^5 , 0×10^3 , 0×10^1 survivors \pm 22V: 0×10^7 - 8×10^5 . (check these for lysozyme).

Note extreme killing (does dilution of the broth increase virulence?)

UV'd PLT22 seems to have lost its protective function, but the dose may have been excessive. Check transducing ability.

C (J) # as above A, FA22 uv 10min. ^{11 hr rough} No plaques at 1, 3, 5, 7.K D. FA22 uv $0 \frac{1}{2}$ ^{11 hr rough} $>$ 10^{-6} 58×10^6 recovery ok!

E no FA22 16 hr rough surv.

(F) 22V UV 10min. assay. (intensity of $\frac{1}{30}$ deleted)at 10^{-5} , C, D, E $\sim 10^2$ - 10^3 survivors with V. Except n.g. owing to high survivors in the control. G.D. indicates that this dose also removed protective power.

Diluted phage is killed by UV much faster than broth, as one should expect.



K: 10 plaque colonies. See L-M for more thorough report.

Any non-plaques by Sogami? 2 lysozymic were not obviously self-plaques in original broth tube there.

B-B

8 "non-contam." colonies streaked out. 6/8 showed 1-5% plaqued colonies and are therefore contaminated. 2/8 showed no overt plaquing. Replicate for further test.

1. colonies evidently not lysogenic. Rebutte, fast whole burst, also ^{ancient colony}

2. " " "

✓ sensitive! all sensitive. Initial scoring as lysogenic - non-contam was incorrect

Conclude that most or all colonies are initially "contaminated".

C-B 8 ^{non-} cont. colonies streaked out. 3 overtly clear.

Replicate

end-to-end, apparently pure lysogenic (might have been up to 2% of the 3 "clear" piles possible ^{relys.} for which.)

2 single colonies not sens. No suff. lysis noted in controls.

Thus, multiple infection may give some uncontaminated lysogenic clones

B-A 15 tests 12/15 single LP22^S & are not 22^S (try 22V?)

1 self-plaqued. [→] all lysogenic (reduced?)

C-A 8 tests: 7 22^S 1 22^R → two out lysogenic (reduced).

∴ There is too high incidence of non-lysogenic. Some infected cells → pure sensitive clones? Save B4 and C1 as 1009BA and 1009CA

1009 BA, 2A - seem pure on streaks.

G. hyrogenicity is a typical "hydrogenated colony"

Transductions - by coaggregation -
protection vs. 22V

1009 L 7

also UV'd FA22

1/1/53

L. Add 10^8 FA22 to 10^4 LT-2. Resorb 10 min. Plate out to count incidence of lysogenization. (assume stocks = 3×10^{10} PLT22)
Also plate ~~_____~~ + $22V$ for purity of these solutions.

M. No FA.

N. as L, UV 500 seconds.

L. 1ml SW414 + .2 ml FA22 ($\frac{1}{30}$) 9:45 -

M " + 1ml "

N " + 1ml FA22 ($\frac{1}{30}$) UV 500 sec.

A: Plate .2 ml on D/O + M or H.

B. Plate 10^{-5} ml with 22V, EMB...

C. Plate .2 ml + 22V on D/O + M or H

B): L ca 400 878

M ca 800 (note 1:1 dilution)

N ca 7. Plate 1ml directly: ca 100 plaques.

O ca 15

stocks all right; UVd phage does not protect.

The design of this experiment is faulty for using 22V/LT2 rather than 22V/LT-14 in the last step.

(over)

O: (no FA) medium useful?

$D(H_{10})$	$D(N_{\text{eth}})$	$D(\text{O}_2)$
0	0	2

A

L ca 50 v.small ca 300

M 2 ca 300

L ca 10 v.small ca 100

C M

O + 22V 0
 (points background) 4

Important comparison:

LA - MA - LC

Note MA \gg LA although 5x as much FA was used.

absolute counts were rather high also, fraction of FA used!

Transduction by 22V may be ignored.

$$\text{Survival of transductors} = \frac{LA}{LC} = \frac{248}{113}$$

$$\text{Survival of population: } = \frac{\cancel{400} \times 10^5}{\cancel{10}^9} = \frac{4}{100} = .04 \quad \frac{878 \times 10^5}{10^9}$$

$$\text{Expected surv} = \frac{0}{B} = \frac{10^8}{4 \times 10^7} = \text{ca } 2.5$$

ca 12/31

SW414. 9 dec UV (ca 400 plaques/plate).

Gal 13 plates
Xgal 5
Hal 10 "

1 mutant Gal- (SW950) and Hal- (SW951)

SW950 also had some "fuzzy" colonies as well as claret ~~plaques~~.
These \rightarrow pure +.

∴ SW950 = SW414 (LT-2) Gal - .

SW951 is slow +, not suitable
as sensitive marker although
uniquely scoreable

Response of SW950:

A Spont Rev: 1 + colony on no spread plate

B + FA22, 1 ml $> 10^2$

C + FA22 (1:30) see 1009 LM 23 papillae

D + FA22 UV (1009 N) 9 papillae + 59 plaques.

Thus FA22 behaves like FA10, 12 in response to UV.
UV'd phage fluoresces, (Cherry reactivity). though it does not infect or poison
All Gal + stable.A: L_p^3 B/C: 20/20 L_p^+ D: 9/9 L_p^3 It can be inferred that FA22 can also
be UV'd so as to separate phage from
transduction.SW950 / ET403 Gal. In \approx 15 plates, 1 sectorial \rightarrow SW952.This is slow +, like 951, and may be suitable only as an unmarked
marker.

Absorption

1011

1/14/53

A SW950
B 694
C - 948.

(3x)

add 10^3 PLT 22 (in 0.1 ml) to 1 ml heat-killed broth (10^9)
9:00 - 9:20. Plate 10^{-1} , 10^{-3} , 10^{-5} & SW950.

- A: ca 20×10^5 ~~discrete~~ at 10^3 , barely confluent at 10^{-1} .
B: ca 100×10^5 at 10^1 , ca 100 small sharp plaques.
C: ca $60-80 \times 10^5$ (mixed) not discrete at 10^3
D: ca 100×10^5

694 lysogenic for LT-2?

Unfortunately, plates mixed.

Consistent with $950 > 948 > 694 > 0$. Rates seem unaccountably low.
*absorp.*Has antigen been destroyed? Perhaps should use live cells for adsorption, then kill. Each killed resp. aggl. (slide) is XII IX serum.

Adsorption of PLT-22

1011

2/2/53

overnight broth cult. Boil 10 min.

Add ca ~~ca~~ ⁴ 10^4 FA22 in .1 ml to 1 ml 10^8 killed bacteria.

Romntryp 10 minutes. Add 1 ml 10^9 ~~sway~~ ^{sway}. Assay (.1 ml, ~~.01 ml~~)

Assay .1 ml

A	LT2	126
B	694	344
C	948	81
O	-	270

Expt. n.g. Use higher densities.

~~FE~~ 1/4/53.

sw952

~~37°~~ 4:40 - 5:10 Normal 10^{10} B + 10^9 PLT22./ml.

- A. Assay initial Bact. 10^{10}
- B. Assay infected Bact. 10^{10}
- C. Assay infective centers B. 10^9
- D. Assay FA 44 surv. A $10^7 - 10^8$
- E Assay FA 44 surv. B 10^9
- ✓ F Assay Gal + A 1 ml
- ✓ G Assay Gal + B 1 ml .05 ml .01 ml
- ✓ H Assay Gal + B + FA 44. 1 ml ... (used 46 by mistake on Gal plates)
- ✓ FF - HH same as O/meth)

$$A 43,38 \times 10^8 = 40 \times 10^8$$

$$B 46,38 \times 10^8 = 42 \times 10^8$$

$$C 34 \times 10^7 \quad \$ \times 10^7 \text{ poor plate} \quad (\text{dimorphism of plaques: spread cont'd.})$$

$$D 114 \times 10^5 \quad 4 \times 10^7 \quad > 10^3 \times 10^3$$

$$E 14 \times 10^7, 13 (+45) \times 10^7$$

Actual initial conditions: Bacteria = 4×10^9 PLT22 = 3×10^8

Bact 22 survivors: s phage = 1×10^7

c phage = 1.4×10^7

i.e., protection was not achieved. Note conditions: absorption in
dilute washed suspension! (Adsorption?) Platings of B showed
1? contaminated colony in two plates (output ca 10% = 8). Test by
replica plating.

Also note: plaques in C are of two types, 25 clear and 13 turbid.
(over)

check plaque type as LT2

22V1-4 : all lytic, similar action

1012B suspension : all turbid

Stock PLT-22 : turbid

2-clear plaques : lytic

2-turbid plaques : turbid

Especially in view of 1012B, clear plaques must have been contaminated (impurity, flamed agaroder?)

Rylee tests of B: 1 lysogenic colony on two plates.

F	4	(FA43)	2
G	9, 11 11 (.1)	.02	.05
H	5, 6 (.1)	5 (.05)	5 .02

Overall result is almost negligible transduction or infection in PLT-22.

(D-meth)

FF	1	4	+ FA43	5
GG	11	156,	.05	58 .02 ml: 34
HH	the same.	160		.02 ml: 40

v. effective transduction: This total seems very high!

Apparently phage was insufficient. All transductions were presumed, but survivorship is obscured. Also note that transductions deficient in colicins may have been too many.

SUMMARY. ~~All~~

$$\text{Bacterium } 4 \times 10^9 \text{ PLT-22 } 3 \times 10^8 \text{ } 22V^R 10 \times 10^7$$

$$22V^R (\delta \text{ phage}) = 4 \times 10^7 / 4 \times 10^9 = 1\% \text{ (cf.)}$$

	col +	Fla +
SW666 + FA46	- 1	-
928 + "	- 1	-

Host adaptations

1013

1/8/53 X Y
 of ., or SW666; SW50:
 FA21, 22, 41A, B, C.
~~22V~~. 43, 46

FA41 = FA21 plaque resolution on LT-2.

	X	Gal X	Y	Gal Y
FA22 - A	50×10^1	7	$\approx 800^{++} \times 10^9$	$\gg 10^{-2}$
FA21 - B	100×10^7	0	$\approx 50 \times 10^5$	1
41A - C	100×10^3	0	$> 100 \times 10^7$	60
41B - D	50×10^3		100×10^7	50
41C - E	20×10^3	0	100×10^7	
43 - F	10×10^1 (turbid)	(12)	500×10^7	lysed. $10^{-2.3}$
46 - G	100×10^7 (turbid)	0	300×10^5 (clear)	0 lyzed

log ratio, essay on Y/TX.

A	adapted to	Y	T
B		X	- 1+
C		Y (x)	4
D		Y (x)	4 +
E		Y (x)	4 +
F		Y	7
G		X	- 1 +

The adaptation of PLT22 to SW666 is therefore reversible, and is presumably a host-induced adaptation.

It also affects 22V, which gives ~~tight~~ turbid plaques on SW666 when adapted but does not induce lysogenicity.

Note that FA22 has a differential of 7 while the adapted phage FA41 have a differential of 4. This suggests a ~~real~~ effect, part reversible, part not (host-adaptation; mutation). This may also account for previous impression that host adaptation was not reversible.

↓ Purify, test lysogenicity → pure Gal+ all show some lysis interaction with SW50! (suggesting that 50 is lytic for 666!).

Transductas key lytic variant

1014

1/3/53

FA46 → X 666 Probable lysis. No T or S.
(FA45/SW618) (long)

A. 928: 4 plaques in 3 plates; no swarms.

Isolate 3 of the plaques. Also pick empty nearby agar. Test on LT-2
#2 and 3 or. show typical PLT22 plaques. #1 did not.

1/9 Retest isolated colonies: all lytic (PLT22 type)

see 1017,

FA43 = 22v/LT2 FA44 = 43/SW950 FA45 = 43/666 FA46 = 45/618.

1017: FA43 → 953 → H+

And back see FA44 inverse of H+ from 22 → 950.

1006 FA43 → 435 → Tr⁺
salt but low yields.

#

- see also 1017

Persistence of H_1^{i*}

1/8/53 FA 18-x SC 666 see 971D18.

4 are i, L_p^s . a, b, c, d.

Prepare FA48 = FA22/a - d. save cultures under these
numbers.

1/19/53. Plating ≈ 300 PLT2 + SW954 ± FA53

+ FA53 \approx 300 plaques, all clear.

100N , 1 ml \rightarrow confluent lysis on part of plate; clear plaques on remainder
 $+ \frac{FA53}{2 \times 10^{-7}} \rightarrow 0$. \therefore same geni tive particles are working.

Prepare S3A = FA53/LT2. Plaques are very small, scarcely discernible. Titer 10 or $10^4? \times 10^6$.

D) 1-2 resistant \Rightarrow SW954/FA53 + PLT2

954 + FA53 seems to show some spotty, ^{general} lysis. Best to separate possible components.

E) SW954, 956 \checkmark not lysogenic / 950.

Some very vague interaction of 950 \rightarrow 948, 956?

F) 954 ~~Lpt~~ (PLT2) showed no lytic response to FA53A or S3A + PLT2. (v.s. inhibition of growth or spreading noted).

& Erow FA53 A (S3/LT2'). Titer (SW950) 64×10^8

January 15, 1953.

Amputates received (duplicate) 1/14/53 as #1, 2, 3 respectively.
(described by Little and Spies' ~~can~~ ~~can~~ canavatian as "cosmopolitan"
Salmonellaphages Label FA 51-3-mp.

a) T test on urine prototypes. All behaved alike except as indicated:

SW950	S	all lysed clean (exc. one rough?)
666		
948		SW948 had background regrowth to all 3.
927		
927RI	+	SW688 and S. coli 3 showed no response to any.
4901	S	SW900 had considerable background for # # # 2.
SW688	R	
550	S	
S. florida	S	927 rough has morphology <u>very</u> rough transaged both.
coli 3	R	
London	S	

b) Plate 1 ml #3 as acid in SW66, 950.
no pili.

SW66, ~~550~~,
& #3 in motility
no swarms

c. Select resistant in patches of both mixtures. Elongates noted:

1. LT-2/51
2. 948/52
3. 750/52.

Tests: 53^R FA22, 44^S !
53^R " " "
53^S " " "

C1 was noted to show full lysis where streaks from 1/53 adjoined 1/22/ bestest. Zymogram of mixture of FA22, 53. Note also resistance to 53, sensitivity to FA22!

Repeat ✓ C1 is lysed by FA22 + FA53, not separately!

a) Obtain C1 lysogenic!

C1 = SW954

C1 and C2 remain serologically smooth (IV, XII); (I ~~XII~~ resp.)
C2 is not sens. to PLT22 + FA 53.

1/13/52

A. Titrate FA22 (stock) on SW950, SW953 in EM13Gal, D(H), O(H)

Titration is recommended for further studies.

1009LB showed FA22 to have activity of mo. $\frac{1}{1} \times \frac{1}{130} \times \frac{1}{10} \times 1000 =$
~~270,000/ml.~~ ca 100,000/ml.

(also 22V, and WV 22 and check transducing for Gal, H-).

1012 GB showed $\frac{1}{1} \times \frac{1}{130} \times \frac{1}{10} \times 1000 = 50,000/\text{ml.}$

of ca. 3000 previously assayed in SW435.

FA22 D(meth) cells have to be washed for D(Hist)

SW	.001 ml	30	(count may be ca. higher - plate somewhat
950	.005	>100	smeared) $\approx 30,000$
.01			
.002	0	6, 8	

FA47.1ml >30 smeared sis!

SW953	.005	3	seems real transduc! for
FA43.1		<100	lysogenic variant. 8 tested, app.
FA44		2	
FA47		9	lp ⁺ normal.

~~EM13Gal~~
EM13Galactose

SW950	FA22	.1, .05	>100	
		.02	.154	ca 7500/ml.
		0	5, 2 (small, elongated,	
1009N/			8+3 delayed	25 plaques
11 ml.			10+3 "	21 plaques

Thus H+Gal+ = ca. 10:1 for SW950.

SW953 has relatively poor response

(over)

D/meth) plates:

SW950 - 7

" +.1ml bath 8

+ FA47 ca 20 (min.)

+ FA47B 10

// + FA52 . 8

(presumably residual FA22)

Proteins - Transduction

1017

January 16, 1953

A. B = SW950, 4 hour culture.

B. B 2 ml + FA22 ("10⁻¹⁰) .2 ml

C B 3 ml + " .1 ml (H₂O) ~~(44)~~

{ 4.1011 8:35 PM - 8:55
- 9:10

R.T.

As assays involve a dilution of FA22 1:300 and 1:300,
salt. is only practicable for A, B.

1. ✓ A, B, C at 10⁻⁷ for survival

2. ✓ A, B, C at 10⁻⁵, 10⁻⁶, 10⁻⁷ with FA44, 10⁻¹.

3. B, C at 10⁻⁶, 10⁻⁷ for plaques on SW950.

4 ✓ ABC .1 ml O(meth)

5 ✓ ABC .1 ml O(meth) + FA44

6 ✓ AB .1 ml EMBOgal

7 ✓ AB .1 ml EMBOgal + FA44.

A)
1 106,130 x 10⁷
2 ca 1000 x 10³, 28 x 10⁵
3
4 8,7
5 2
6 0,0,1
7 1, (lysed)

B) 93,100 x 10⁷
78,87 x 10⁷
1425 x 10⁶, 1000 x 10⁷
> 10² est ca. 3x difference
> 10²
19,24
56,85

C) 107,88 x 10⁷
3,6' x 10⁶, 0 x 10⁷
ca. 1000 x 10⁶
Phage ridden! 4,4
2,2
10⁻¹ plaque ridden? 2,1
0,1 lysed

8) 1009N + 1A: e-, ca 10 plaques. 694 H+ colonies: tet8 for gp⁺; all are Lp^s. Define transduction by inactive phage.

Note excessiv phage in 3B, 3C

lysogenate in 3C!

Summary: initial bacteria: 1.2 x 10⁹ + PLT22 (B). 97 x 10⁷ C) 1.03 x 10⁹ (possibly samey will but uncertain)
FA44 SURV. 2.1 x 10⁵ = ca. 10⁻⁴ = .33 x 10⁹ 5 x 10⁶
= 85% = 1/2%!

Add PLT22, calculated 10⁹ →

.3 x 10⁹

Why discrepancy between B and C?

(see over) C was allowed to progress only slightly longer, but FA44R is 50x too low.

Accessory mamp. phage

1018

A = .02 ml FA22 EM180al; (.02 ml D/meth)

B = 1 ml FA47B

C = 1 ml FA43

D = SW950 .1

E = SW955 .1

		col	D(meth)
1 - 11	D'	.	1
2 - 12	E'	.	4
3 - 13	D' + A'	"	26,20
4 - 14	E' + A'	"	0, 6
5 - 15	D' + B'	"	4,
6 - 16	D' + B' + A'	"	13,27
7 - 17	E' + B'	.	3
8 - 18	E' + B' + A'	"	10,7
9 - 19	E + C	"	"

q. 3/6 no accessory.

4/8 ? low yield.

Cetrimidine

1/21/53 Cetyl TriMethyl Ammonium Bromide (Cetrimidine). rec'd from Dr. Gorn (Baker lot # 1.1446 - opened.) Acc'tg Bradley + Boyd inhibts phage adsorption.

Mix 10^4 PLT22 + 10^9 ~~swiss~~ SW900.

Add .1 ml successive dilutions of CTMA, plate .1 ml.

O.

1.	.1 ml 5% =	$5/1000$	$\approx 10^3$ bacterial survivors
2.	$\times \frac{1}{5}$	$\frac{1}{1000}$	" 10^4 ; occ. plaques
3.	$\times \frac{1}{5}$	$\frac{1}{5000}$	$\frac{1}{15}$ perhaps 50% endpt. count;
4.	$\times \frac{1}{5}$	$\frac{1}{25,000}$	$\frac{1}{125}$
5.	$\times \frac{1}{2}$	$\frac{1}{50,000}$	
6.	$\times \frac{1}{2}$	$\frac{1}{100,000}$	
7.	$\times \frac{1}{10}$	$\frac{1}{10^6}$	

} full count

Not useable for limiting cross-infection.

Diploid Hfr crosses
further commentary 6/10/53

952. (Hfr lac⁻ x Hfr P⁺), Abandon → H316 Mal-lac⁻ TL-
W1940 W1590 see 960
F status? (Pheno. Elimin.)

953 H312 x Hfr → Malv lac⁻ SV TLR M+ No pure Mal⁺
F⁺ H267 Mal⁻ H318, 319. (self eliminating?)
(2n F⁺ x Hfr) Held

F status? Elimin.
if standards?

955 H310 x 1895 1/2 Malv. H313.
6/24 (Mal⁺)

But appears to have segregated already in previous expts.

H310 then noted to give very high yields x F⁻
H310 segregants: 2 each lac⁻, lac⁺ are F⁻. Test
more extensive pools. → Then 10+, 5- all F⁻.

956 H311 (Hfr 2n) x Hfr. No Malv. Recursion ~~not tested~~ not
perfected 1 Recursion test.

23 lac⁻ tested: all Mal-! (Very few Mal⁺, but
some found).

Abandon all these.

Continue H310 x

Hfr lac⁻ x lac⁺ ... (1940 x 1953 / EMS lac...)

958. H245 x W1922. Some Mal^{lacv}
Hfr λ F⁺ Hfr.

Hander

Hfr F⁻
964 H310 x W1607 nEMS lac. $\frac{1}{2}$ Mal^{lac+}
Mal^{-SR} Mal⁺

→ 3 lacv. 2 Mal⁺ 1 Mal⁻ (H322)

need hemizygosity test! (note H310 is Mal⁻).

superseeded by 1057

July 4, 1952.

Summary of Hfr x diploid crosses etc.

952. $Hfr \text{ Lac}^- \times \text{ Het } \text{ Lac}^- \xrightarrow{\text{EMS Lac}} \text{ occasional } \text{ Lac}^v \text{ (balanced)}$
but mostly Lac+ recombinants. $H316 = \text{ Mal-Lac}^v \text{ TL-}$

should be crossed
 $\times 124$
 $\times 161$

953 $(H267 s^R \text{ Mal}-) = H312 \text{ prot.} \times W1895 \text{ in EMS Mal.}$
not ready yet $\rightarrow H318, 319^{7/1}$. ~~should be segregated~~

✓ Mal+ are TLB-. Cross - MalV

(mostly Lac+, Mal+).

955 $H310 \times W1895$ (1895×1177 nardiaj. TL-Mal-Lacv) EMS Mal

In first trial gave $1/4$ Malv $H313$ Lac+ Malv S^v

second: 0/24. But $H310$ itself is Hfr! Mal+ Lacv?

Rechecks Hfr x F- \rightarrow F- (haploids) only.

prot

956. $H311 \times W1922$ (Het diploid Lac-Mal-Malv prot). EMS Lac.
gave numerous Lac+, 6/6 dip pure Lacv Malv Mal-S^s
Rare Mal+ 5/5 Mal+ Lacv

Further tests needed, but presumably may mean that elimination is
not bypassed in $n \times 2n$ (still n for Mal).

$H320$

A: Mal- Check for hemizygosity

B Cross s.g. parents

958 H245 x W1922

TL Lacy Mal-

EM8 Mal. underway

Malv isolated. Test progeny for resistance, Ifc.

H321

960 TL ^{B4} H316 x W1895 EM8 Mal. Chuck Lacy Mal - for
tested only on Mal! breeding purity.

959 H245 sec. Continue

To be done

- a) Further tests on ~~H315~~ 955: for lac^r Mal-pme (is Mal ever eliminated?). H310 itself may be hemizygous?
- b) Segregation of H313 \rightarrow recovery of Hfr?
- c) Transmission of Hfr to Mal⁺ progeny?
- d) Transmission of Hfr in H316 \times Hfr? etc.

959. H295 secundarius: H317 $hp^+/-$. hp^2R segregating?
obtain Mal⁺ reversion for coupling; regulation.

954: lac^r δ^R recomb from F+ \times F-. Checks micro machines to Campephatum with 1851 \times 1956

957. What peculiarities of $\lambda/1827$ can be demonstrated?
In gross test, λ^Y did not occur.

Phase variation of Kunyendorf

1020

2/22/53

Kunyendorf 6145-52 = A Ξ . single colony picked and
motility = AI. = SW961

list of serums:

- 1 Kunyendorf
- 2 melneaste 240
- 3 beltin
- 4 novomiris 20
- 5 para A 228 new
- 6 mosehead
- 7 para A 228 old

2/22. Inoculate A into serum tubes:

k1, k6.

After 48 hours, k1 showed rough
blobs which spread very slowly; 2/28: spread through.

k6 grew out and filled the tube = A/k6. Repeat + test antigens.

On slides unpurified A/k6 = B_1^1 (ants c $\leq 1,5(k1)$) but not 1,5(k6) or 1,2
positives are quite weak.

(over)

2/22 Inoculate AI on plates of k1, k6 agar.

A 2/3: bulbs on k6, not on k1 - slow or no spread. (surface spreading)
P 2/3 pick \rightarrow 0. $= E$ (inches plates less spread may obscure blobs. satisfactory than tubes)

A 2/4: numerous B's on small k6 plates. 1 small rough bulb on k1. Remember to try to pick later. for prolonged incubations.

C = cholera suis c: - #153

D = AI/k6 plates (4 serums / 3 small plates).

If mass cultures all react c: + k1: + k6 - in slide

tests. ~~Test~~ use D 3 as strongest reaction and test single colonies.

Each of 4 colonies react c+, k1+, k6 - in slide (= s)

Prepare broth culture for titration. Grow D 1 in k1, k6, E: grows promptly through k6 is slightly inhibited in k1, strongly inhibited in c but definite diffuse spread. 2/28 still not spread.

E: one plate showed two rough blobs. Pick to reassay. (May have undergone two steps of selection)

sw

D1 = 958

B1 (crys.) at 1:500 C KI 46

A1 - +F *

B1. 1:100 ++ + -

(✓ overnight : same)

separates fairly char.

1:500 overnight ++ + -

D: Mix in k1, k6 SS. transfer k6 overnight; k1 in 48 hours = D2

2/26 D3 Mix D2 with k1

Titrate D1, living & heated 58° 1 hour & formalized.

living	C: 1000 +++	k1: 50+++ 100++ 200+ 500-
heated	C: 1000 ++	k1: 50- 100-

Repeat

living	k1: 100 ++	k6 : 50 -	-
form	: 100 +	: 50 -	-
heated	: 100 -	: 50 -	-
D2	living	k1: 50 +	k6 : 50 -
E1	"	k1: 100 +	k6 : 100 -
E2	"	divide	C: 1000 + (poor) ++

~~Repeating for D2...~~

carries notes of humzendorf = 1,5, x... : C, x.., with x-component absent in other 1,5 sera. (cf. Berlin serum). Heat lability makes sonatic antigen unlikely, but reactivity of living cells is much better than formalized. Same type is secured by selection in humzendorf serum.

Titrate Berlin serum : D1 living

1:50	100	200	500	1000
+++	++	++	+	÷

∴ Berlin is
even more satisfactory
some (titres 1:500)

2/23-1 B1 (not pur.) into tube of k1, k6 SS.
A1}

overnight:

A1	<u>k1</u>	<u>k6</u>
B1	slow diffuse spread	2 v. small bulbs. ++ near end of tube

2/25. Titrate D1, A, C: (1 hour 37°).

A. ~~C~~: 100 - 500 - <100
 k1 : 500 ++ 1,000 ++ 2. ++ 5. ++ 10. + 20. + 1: 10,000
 k6 : 500 ++ 1. ++ 2. ++ 5. ++ 10. ++ 20. ++ >20,000

C: C 1,000 ++ 10. ++ 100,000 - <100,000
 k1 100 - k6 100 - <100

D C 1,000 ++ 2. ++ 10. + 10,000
 k1 50 - 100 - 200 - 500 - 1000 - 2. - <50
 k6 50 - 100 - 200 - 500 - 1. - 2. - ! <50

of B1 at 1:100, 1:500! 4 hours: ib. (± k1 1:50, 1:100)

Rx to C ++ k1+. still shows in slide aggl. form colonies from streak of D1. Cf. heat killed cells (sandwich component?)

Kenyendorf - 958
further fixation

1020

2/27 G-tive cells and heated in bovine serum 10 mins.

	C 1:100	bulin 1:500	bulin 1:100
SW 958 liv.	++	+	++
heat.	-	-	-
choleracaeusis 153 liv.	++	-	++
D 2	++	-	..
liv. { E 1 prifit	++	+	++
E 2	++	+	++

∴ factor is also present in 153, presumably selected by a van Kenyendorf serum. It has been substantially eliminated in D2. The factor is presumably absent from diphasic choleracaeusis which migrates readily in Kenyendorf serum. It is heat-labile (presumably H-), but also formalin-labile!

Test serums at 1:50 with D1 living

bulin 1:5 (k3) and ⑤ (bulin absorbed c 157) ++++
 all others negative: k2, k4, k6, k7, 1,2; liv, env, d., 1,2,3 (colindale)

To select C or D phase from C:1.5, presumably should use 1,2 serums.
 "phase stability" of javiana may be due to similar cross reactions of C and 1,5.

Effect of formalin. (Add. 5% formalin to D1 cells)

	C 1:1000	bulin 1:100
liv.	+++	++
form.	+++	-

	C 1:1000	berlin 1:100	T Vi	1:50	C 1:50
SW 958 I.V.	+	+++	-	-	-
SW 961 I.V.	-	+++	-	-	-
D3 (not pur.) (slide negl. 153)	+++	-	-	-	-

c' factor is evidently absent from Colindale c.

Further questions:

- ① Does this explain all anomalies (cf. 45-47).
- ② Recession of c
 - a) 958 appears to be stable in c (Colindale) SS. cf. D3.
 - b) does Colindale c, whilst reagglutination of ~~SW~~ 961 + tested 2/18 no.
- ③ Is c' present in Edwards' c serum? (not previously detected with 1,5, c'... owing to formalin-lability.) If so, c' might possibly best prepared by absorbing cc' with D3 ~~or the heated c' cells~~. Absorption of berlin serum with, e.g., para A 1,5 would be less safe.

Misc. tests:

3/1 E. 153 ^{somewhat} restrained in berlin serum (verify c')

SW 961 is not " " c (Colindale).

Titrate in c, berlin:

(mono. c-) 902
D3 pura. 958C

⑥

SW 958C

26 (slide negl.)

958 -

c 1:1000
++
+++
+++
++

1,5, c' berlin 1:100
—
—
+++

961 ++

3/4/53. Stability of c phases:

F)	hoc c: SS	SW958	3/7: no	3/10: 3/13	3/15
	3/4/53.	958C	mobility unknown	idem	idem
		#153			(mobility perfect & stable throughout)

Other serums: SW961 in SS:

- 1. 1,2,3 bins - 24-48 hours swam through
- 2. k5, k7 (para A 278) - 2-3 days " " (k5 slightly slower)
- 3. k3 - substantially immobile in 4 days: rough buds.
"1,2,3" serum probably purified
(try Edwards 1,2. if no!)
- However, k5 and k7 are "affluent serums", may still have some residual components (agg. titre < 1:50)
- 4. 961 / 1,2E (157 serum): swims about equally prompt in k6, 12 Edwards, 1,2,3.

Stability of 0-901

1021

2/27/53.

Fresh stocks of 0901 received from A. Fabix. Also test SW 542, an adult, and SW 506 resuscitated from lyophil. (0901 from Kauffmann and Edwards respectively).

In feb., 0901 Fabix 1-2 remained stable 4 days. On plates, (1 each) #1 stable; #2 gave 1 swarm 48 h., 2d 72 h.

A: 0901#1 0 swm. 1st. 2d plate: 1 swarm: both (d)

C SW 542 0 swarms 1st plate; 2d plate: 1 swarm: (d)

D SW 506 1 swarm : (d)

∴ All cultures are about equally stable (ca 1 swarm / 2 plates)

E 0901#1 + FA 22: 8 swarms after 24 hours (control 0)
v. numerous trails 48 h. 1 all (d)

F + FA 9 (SW 663) 3 swarms several trails
+ FA 12 (SW 628) 4 swarms several trails

0901 + F19.

10/13

~~Has 5 tubes each \$5 WB, W1678.~~

~~overnight: motility not diffuse but from coelocent swarms fresh.~~

C - D₁ single colonies as above.
WB W1678

C1-3 "smooth" 4-6 "rough".

10/14 overnight:

C 1 spreading is initially rough and patchy; later diffuse from

2

3

4

5

6

"

D

1

2

3

4

"

"

10/27 ^{Reinubate} All marked bottles except D4

Second passage: all marked bottom, ca 24 h.

A. 3/2/53 Strain out on N.A. Purified isolates to HLB compatibility test

	* 1878	1958	①
C 1	+	+	
2	-	-	
3	+	++	
4	-	-	
5	-	+	
6	-	+	
D 1	+	-	
2	+	-	
3	-	+	
4	+	-	
C 6-0	↓	+	
D 6-0	-	+	

	1876	1958	②
C 1	+	+	
2	-	-	
3	++	++	
4	-	-	
5	+	-	
6	+	-	
D 1	-	+	
2	+	-	
3	-	+	
4	+	-	

① Grouping both together 24 h
plated on EMB Loc.

0/0

③ by SL on D (a)
A x 1177
B x 1817

all over.

	$\times A$ W1177	$\times B$ W1876		
C2	-	+++		W2207
C3	+++	++	D1-O	W2206
C5	-	+++	+	W2208
D2	+	!		
D3	-	4	1607	C60 C3
C6-O (58-161)	+	+ (+)	D1-O	+ W2209
D1-O (W1678)	++	!	1607	
1607	-	++	++	

D4 ++ ad. ± still F+

~~C3 is High fat.~~

D3 seems to be F- but also sterile.

D2 is poorly fertile, not F-

C2, C5 seem F- !

try D4 now.

1177 x 2209	-
1876 x 2209	20
1177 x 2209 (1876)	40
1817 x 2209 (1876)	20 3
1177(2209) x 1607	-

Reproduction and F test:

needs to be checked.

2209: after grown in 1876

becomes fertile in 1177 and 1607
(but also fertilizes W1177! ?)

2205 fertilizes 1986

Reproduce this test after
purification.

2207-2208 are fertilized by W1876.

F/mot. recaps.

1022

4/6/53.

6 cultures 58-161, 4 of W1678 sent through motility tubes (2 pass.)
3 and 1, resp. were altered.

58-161:

W2207, 2208 : F^- , non-infective, infectable

W2206 F^+ nearly Hfr. Infective. ~~Not~~ Not studied for
nitrate. (see TCN) (found no SR+)
see also 1113

W1678

W2209

F^- , non-infective

3/ Injuro fresh FA (5YA) from ~~pure~~ purified \geq_6 , phase of 3 ga.

3/13/53 P: 5YA - X LT2² < 24h $\rightarrow \underline{d:1,2}$

~~(R)~~ ~~(S)~~ ~~SW999~~

(R) 891 x- / 5YA. swarm overnight. $\rightarrow d$
 (S) 959 x- $\rightarrow d$
 960 x- $\rightarrow d$

Div these broths, unpurified, into d+1,2 for possible 26 phases.

3/18. No alt. phases.

Repeat 3/18/53 using modified 891, 959., in d-1,2 serum.

3/19/53. Swarm in S (in d. T.O.) (d:1,2 tube). Other 1,5 (2 d:1,2 each): no swarm overnight T.O. 3/28.

3/20/53. 1,235 swarmed. 3/21. \geq_6 : - purified, no swarm in \geq_6 in 24h. Send to Edwards as sw999.

sw999 / \geq_6 = 999B: retests \geq_6 ++ 1,2 ++ 1,5 ++
 2- 5++.

∴ new phase is \geq_6 , 1,5... or mixture. Rests like

cf. sw999
 999B (not pur). $\begin{array}{ccccc} \frac{\geq_6}{++} & \frac{1}{-} & \frac{5}{-} & \frac{1}{-} & \frac{5}{-} \\ \frac{++}{++} & & \frac{+}{++} & & \frac{++}{-} \\ \frac{++}{-} & \frac{++}{+++} & \frac{++}{\div} & \frac{-}{++} & \frac{++}{-} \end{array}$

5/5 single colonies

behave same way. sw999 apparently reacts fairly specifically in \geq_6 . Still, moribund in 1,5 SS for further solution.

sw999 and 999/1,5 serum still react in bacilli 1,5 serum at 1:100 dilution are not inhibited by it.

A. SW938 (1,2:enx) \times FA40 (sendai a:1,5) [2; enx serum ss] I

B. " " \times FA3 (attenuated C+1,7) I " I

C. SW676 (z33) \times FA22 (i:1,2). [z33 ss]

D. SW933 (i:enx) \times FA40 [i; enx]

dense bulb but no swarm. 2/28 swarmed though.

E. SW676 \rightarrow LT-2 I (i:1,2) [i; 1,2]. Moderate spread of monolayer in control (adequate). i? 246.

Reinfect: 3/1 no buds continued slow spread: 3/3 all are i; exp. not adequate

F. abny 1+2 \times FA55 (-1,2) } No buds in exp. or control 2/26
G. " " " 56 } 3/3 later slow spread of v. dense bulbs.
H. " " " 57 } all still b recover

2/28 A: controls and test show bulbous but no swarms. } 2/28
B. " " " "

C. control: no spread 3 tests: all swarmed + fibroblasts. Theoretical
C1: i test s.c. in i ss: no buds. See note *

D. after motility test, a: C2: i " " +
C3 (incomplete swarm): still b slow spread (i probably weak)

J. FA54 \rightarrow SW891 (-1,2). Swarm through in 2 hours. 2

K. " 959 " Control immobile. See 1023/4C
L. " 960 " (959 shows v. slow diffusion) suggests all of
these are H, 1,2

2/28 M. FA50 (SW546) \rightarrow manni (6500-51, a:1,5) [a/s] These are H, 1,2

3/2. Slow spread in epith., not control. \rightarrow all react 1,2+ and 1,5- (slide) else #153!

N. 15 (abny b:enx) \rightarrow jirans (732-49) (lev:1,5) [lev:1,2] see 1028

* Note (2)- serum seems to restrain attenuated (1,7) but not sendai (1,5) (+ a serum)

A - failed ? Needs multiple implications

B - " . Probably ⑤ has anti 1,7

C - i:1,2 → \geq_{33} → i: -

save one as 1023C1

best evidence so far
that \geq_{33} is a phase
1 homologue.

D (parallel to A).

" i: env " × a: 1,5 → B: a: env. SW 975 (cf 925
 $= D, a, env$)

PA 40 (nominally phase 2) may be ~~be~~ mixed?

~~check phase 2:~~

F&H: (-:1,2) → abun 6:env 3/7: no consistent spread in any
expt. or control. Activity of PA? 3/10 isolate spreading dense
bulbs.

23F Rep. - 3/31 still b.

G Inapp. → "

H "

SW 973 (3 cultures)

M. 1,2: - → a: 1,5 → 1,2: 1,5 S.c.i all passed through

② serum. → $\begin{matrix} \textcircled{5} \\ \textcircled{2} \end{matrix} \leftrightarrow$ (slide)

M1 in 1,2,3 serum gave turbidity
from restricted growth. 3/7: no serum.

5/6 in ②: immobilized

M (control) eventually swam through:

~~still 973~~

$\begin{matrix} \textcircled{2} \\ \textcircled{5} \end{matrix}$ - maybe 1,10 or 1,11?

1,5 ++ single colonies again

1,2 ++ reacted in ⑤ as did
older broth.

∴ control gave no change

SW 959 eventually gave spread through tube; 891 and 960 remained immobile. magg. b, i, 1, 2. pr ± Replete v magg. But through SS.

3/1 JKL, ~~SS~~ reacted (weakly) in d. Plant single colonies in d serum:

$$\begin{array}{l} \text{J inhibits bulb, } ? \text{ subag. spread} = J' \\ K " " " " = L' \end{array}$$

through d: $\begin{matrix} (2) + \\ 1, 2 \rightarrow (5) - \\ 1, 2 \end{matrix}$

zeta/Z₆ uninhibited spread.

Single colonies of both J and L / ~~had~~ react both in (2) and (5) note L, originally k leaves similarly.

This would suggest

$$d: Z_6 \text{ (phase not necessarily pure)} \rightarrow 1, 2 \rightarrow d: 1, 2 \checkmark$$

J", L"

Test J', L' in 1, 2 SS. \rightarrow ~~—~~ Q. $\therefore J, L$ are d: 1, 2

→ single colonies: J:

(2) ~~++~~ +++ (5) -

L:

(2) ++ (5) +++

SW 960:

+++

+?

Z₆; lew —

in tubes

(2) 1:2000 (5) 1:1000

J'	++	=
L'	++	=
960	++	=
891	++	=

$\therefore (5)$ cross-reactions appear on slides (at higher conc.)

K finally grew out in d serum: maggotinable.

Select in SS: \rightarrow maggl. (j?) K itself lost. save K' (magg.).

J" = SW 974
K" = SW 977
L" = SW 978

v. wt. remains even after passage twice in SS d?

3 phases $\begin{matrix} 891 \\ 959 \\ 960 \end{matrix} \rightarrow$) see 1031.

Hold off further work until \rightarrow —; eng. etc. is understood.

1023 FG & H repeats

3/29: F -

G-H small buds / not progressive
(56, 57 → abn)

G: 3_{33}^3 :
maybe 22^s. Test single clams +
verify 233: env

M. scallaneous ♀ and stability tests 1024

2/26/53 et seq.

	12	22
SW676	++	-
SW546	-	++

Test unpurified
stocks.

A.

938	++	+
altendorf	-	++
0901-1	±	+
SW959	++	+
SW960	+	-
denbare	SW843	-
budbury	SW730	-
bispebjerg	SW725	-
(typus ±)	SW714	++
J. Taylor	abony 133-52	±

a + ~~++~~ 12
b ++ enx ++

injected undiluted
in 1/2 serum
to give 1-
scattering culture
not morophatic
but might be
somewhat stable

B

abony	177-53	-	-
ball 11-50		-	-
f. p. 783-52	+	++	
SW891	++	++	
jess. 732-79	++	++	
denb 130-52	-	-	
schmitz v726	-	-	
52	-	-	
f. p. 1102-52	-	+	
3iga 317	-	++	
shandish 208	-	-	

a ++ enx ++
b - 1,5: ++ 26: ++

cf. stocks:

a - enx - after neutralizing
d . . 26 ++ ± ±

isolate 2 phases
d agglutinates/poly.

C →
→

Stanly	5099/50	-	-
muni	16500/51	++	-
ball 268	-	-	
f. p. 874	++	++	
848 saliv.	-	-	
i.: 890A	+	++	
muni 1885-52	++	-	:
" 3840-52	-	-	:

d ++ 12 ++

a ++ 15 ++

a + enx +++

a + 15 ++

more granular

SW874 stocks: slide 4a: 5 mag.
eggs on colonies + ca 2-3 in.

passage has single ♀, enx
874: colonies though ~~++~~ 5 mag.
gave culture, each egg
nearly both i ♀, enx
too unstable for present purpose

(over for page B)

efabry
(typ 209)

Test 4 type para B stocks for phase purity.

Bottles don't from stocks.

a) slide aggl.

b 12

Jersey

Dundee	++	+
1	++	++
2	++	++

Taunton

++ ++

Burles

++ -

Jersey

++ -

3B

++ + ++

BAOR

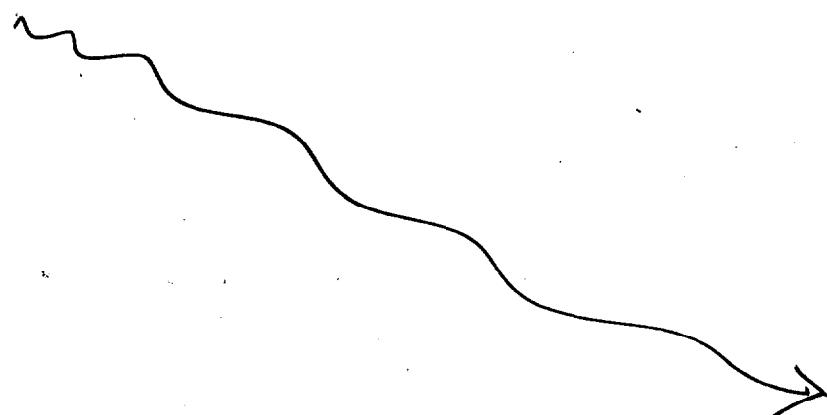
+ + +++

3a F

+ + ++

3a

++ +++



Aepiat

			12	22
-:ens	brandenburg	24	-	-
	ab. equi	26	+	++
	ab. obis.	28	-	-
	" "	29	-	-
D	dublin	65	+	+
-	d.e.sal.	72	-	-
-	para A	2	-	-
	"	229	? -	? -
	wren	281	-	-
	wagene	290	-	-

- numerous faint, tiny plaques!

S. buddingy strains from Edwards (non-XVII)

	PLT 22
1	3807.52
2	4102.52
3	4641.52
4	5435.52
5	5437.52
6	6504.52
7	6612.52
8	303.53
9	517.53

R shaded
S numerous plaques.
shaded

lv: 1,7 Sat 9/86

E

4102 proved to react D:a!
culture is recorded as loma linda.

of previous page: this

F

Tube agglutination:
1 Beede
2 Jersey

b 1,2
+++ =
+++ =

b: --

use b serum to
isolate 1,2 phase for
subsequent hemagglutination
to b:ens: no agglutinins —
these are monophasic:

Notes on cultures from Chamberlain

3/1/53

(Brought back 2/15 + 1/53)

SW 961 = *S. cholerae suis* Koenigsdorf recently isolated, 6145-52, purified and motile. = -:1,5,(c'?), ...

SW 958 = SW 961 selected at 4W / Minnesota serum. = c,c':-

958C = 958 / Koenigsdorf serum. = c:-

Non-motiles: 1520-50 to 1526-51 (see SW 552-553) presumably all nonmotile ~~dubius~~ from outbreaks in Guatemala. See letter to Munro in ca 2/53. ^{1523, 1526} Should not be found. Other NM not selected in accessories. Relationships of 3010-49 to 3012-49 not questioned.

? - Kauffmann's D nm: is recorded as highly aberrant
= stocks ~~2~~ 234?

Monophasics: -1,2 Striae 191: history? listed as typhimurium,
SW 960 = 5594-51 (Kauffmann...) isolated in Berlin. Phage types as pure B but
is -1,2. (Bebek or Baden?)

SW 959 = Himes VA H -? no other label. listed as -1,2

3550-51: 2 cultures found, named monophasic 1,2 and b resp.
works out to name 3550-51 as b:-, 3551-51 as b:b2. [3550-51
is probably a fairly stable b that later did give a 1,2 phase. This should
be verified].

SW 891, acc. to PRE letter, is $\overline{\text{E}}:1,2,3$ (Theil-Cornell) \therefore TM.

\geq_{33} homologues

3/4/53 occurrence: (see also SW. m.)

~~et seq~~ 991H1A = SW937/b $\rightarrow \geq_{33} \leftarrow ++.$

K1A
K3

See 979-1 and 11. J14, K15-16 kept as magnifiable.

Now tested as possible \geq_{33} variants. — not \geq_{33}

After motility test: + (through \geq_{33}) ↓ / through enx

J14 enx, i
K15 enx
K16 enx

i enx
enx: \geq_{33} ? v. weak
enx

enx! \geq_{33} ?
~~enx~~ \geq_{33}
enx

$\therefore K16 = \underline{\geq_{33}: enx}$ & typical variability = SW981

K15 may also counts \geq_{33} ; 1,2 at 1:500/-1:1000!

but strong spontaneous: may be "reduced" T.O.

Note 1023. SW676-x failed. Repeat

3/15.

FA49 \rightarrow SW666 strains.

16 slow buds only, eventually grown out

\rightarrow LT-2 " " dead, as basis for outgrowth.

\rightarrow aborty no mot. at all. T.O. 10/21

ca 4/15. ~~SW1036~~ SW1036 diluted from SW703 / b+1,2 in tube (1/3, after motility test) $\geq_{33}: 1,2$. The ph2 appears by motility selection of ph1.

SW1005 aborty (mot.) | b, enx plates - indistinct swarm.
 $\geq_{33}: enx$ (\geq_{33} verified) cont. Motility \rightarrow enx.

Cross-enx i b+12

presumably parent

medically visible in

b. Not especially
in i:1,2,3.